A DIURNAL ACTIVITY RHYTHM IN PLETHODON CINEREUS AND ITS MODIFICATION BY AN INFLUENCE HAVING A LUNAR FREQUENCY

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It has become increasingly clear that many of the physiological processes in organisms do not occur at constant rates, even when the organism is in a constant laboratory environment. These fluctuations in rates are often of regular recurrence and may be designated as rhythms.[2] Various manifestations of these changes taking place within the organisms may be observed. Among them are rhythms of O_2-consumption and CO_2-production, locomotor activity, chromatophore pigment dispersal and body temperature changes.

Judging from the number of contributed works in the field of biological rhythms, locomotor activity has been more often utilized as an index to rhythmic behavior than any other kind of biological process. The simplicity of automatic recording devices needed, the long span of time over which animals may be used for such studies, and the minimal interference with the animals' normal functioning, are some of the reasons why activity studies have been so popular.

Among the earliest investigators of activity rhythms was Szymanski (1918), who demonstrated them in a variety of animals. Subsequently, studies of the activity rhythms of a host of animals have been made. Among them, to name only a few representative ones, are those of Ralph (1957) on the earthworm, Harker (1956) on *Periplaneta americana,* Brown (1954) on the oyster, Marx and Kayser (1949) on lizards, and Aschoff (1952) on mice.

The diurnal rhythm is the one most commonly encountered in terrestrial organisms (reviewed by Welsh, 1938, and Kleitman, 1949). Among marine organisms both diurnal and tidal rhythms have been found associated together (Brown, Fingerman, Sandeen and Webb, 1953), but sometimes a tidal rhythm may be the only one apparent (Rao, 1954). Lunar periodicities in marine animals are well known (Korringa, 1947).

That lunar influences may also be significant in the metabolic rhythms of terrestrial organisms has been indicated by recent works (Brown, Freeland and Ralph, 1955; Ralph, 1957). The present study was undertaken in order to examine the activity behavior of a terrestrial animal, the salamander, *Plethodon cinereus,* and to analyze it for lunar influences.

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[2] A rhythm, as used here, is defined as a definitely persisting, regularly recurring, quantitative change that continues after external stimuli are withdrawn. Rhythms can be roughly divided into (a) those of 24 hours (diurnal) or less, and (b) those of more than 24 hours (*e.g.,* lunar). They generally have a causal relation to external factors, but are to be distinguished from periodicities which are of extrinsic origin and which vary directly with environmental factors.

Experimental Procedure

Twenty-four adult *Plethodon cinereus* were collected on May 8, 1955, in a beech-maple forest near New Buffalo, Michigan. The animals were brought to the laboratory and placed in two 9-inch crystallizing dishes, the bottoms of which were covered with a moist sand layer and bits of wood debris. Small earthworms and sowbugs were provided as food and these were replenished frequently during the experiment. The dishes were placed in a slowly-moving stream of cold tap water.

All work was carried out in a room designed as a photographic darkroom. There was one opening to the room, a door fitted with a light-baffle. Thermograph records of the air temperature a few inches above the running water in which the animals were kept throughout the experiment were taken between May 10th and 26th. These showed no diurnal temperature variations, but rather only slow changes requiring several days.

Continuous records of the locomotor activity of seven animals were obtained by the use of the same apparatus, and data were translated from the experimental records to tabular form in the same manner, as described by Ralph (1957) for the study of the earthworm. The recording device consisted essentially of seven rectangular platforms that rocked freely upon knife-blade bearings. Upon each platform was mounted a chamber consisting of a 3¾-inch Petri dish enclosing the bottom half of a 2¾-inch Petri dish, thereby forming a circular track one-half inch in width. Movement of the animal around this track resulted in different degrees of tipping of the platform. Platform movements were transmitted via a thread to a recording pen system that reproduced them on a sheet of paper moving at a rate of two centimeters per hour. The recording apparatus occupied a position adjacent to the dish of reserve animals and the activity chambers were suspended about two inches above the water's surface in order to keep the animals in the same cool air layer.

From May 9th to 13th all the animals were exposed to alternating light, from 6 A.M. to 6 P.M., and darkness, from 6 P.M. to 6 A.M. The light source during this period was two 7½-watt opalescent, incandescent lamps suspended about four feet above the animals. The form of the diurnal activity cycle under simulated day and night conditions was determined during this period.

During the afternoon of May 13th a light-proof box was mounted over the water table in which the reserve animals were kept so that they could be maintained in constant low illumination. The box was equipped with a light source at the top so that diffuse light of less than one foot-candle reached the salamanders. Also, the transparent glass covers of the activity chambers were replaced by black-painted covers in order to exclude all light. Thus, when the animals were in the reserve dishes they were under continuous and constant low illumination, and when placed in the recording apparatus they were in darkness. Twenty-nine days of continuous records for seven animals at a time were obtained under these conditions.

A regular sequence of replacement was established at the outset of the experiment, so that only one or two animals were replaced daily. As the animals were removed from the chambers they were placed in one of the crystallizing dishes, while the replacement animal was randomly selected from the other dish. When the supply of animals was exhausted from one dish, replacement was started from the dish that had been receiving the animals. Thus, each of the 24 animals participated in the study at least twice for approximately four days each time.

In order to minimize any effects that the placing of a fresh animal in the apparatus might have on the data, the time of replacement was varied over the day from 8 A.M. to midnight. As a further precaution, the first three hours of the record produced by a fresh animal were not included in the data, since the animals tended to be hyperactive for several minutes after being placed in a chamber.

Results

Diurnal rhythm

The mean activity for the animals in alternating light and darkness is shown by Figure 1, A.[3] It will be seen that the animals were most active during the

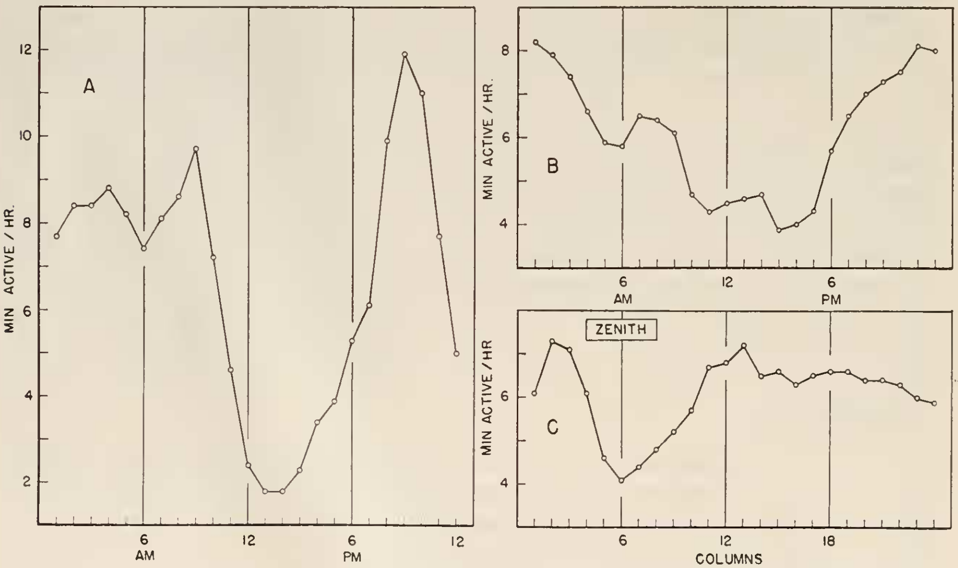

FIGURE 1. (A) The average activity of seven salamanders for about five consecutive days while exposed to light from 6 A.M. to 6 P.M. and darkness from 6 P.M. to 6 A.M. (B) The average activity of seven salamanders for 29 consecutive days. Their activity was recorded while they were in darkness. (C) The influence of the lunar-day frequency on the activity of the salamander, as demonstrated by analysis for lunar effects. (See text for explanation.) The times of zenith for the 29-day period were synchronized in column 6 for this analysis.

dark hours and least active during the light hours. A more complete analysis of the exact form of this curve will be presented in the discussion.

Table I shows the mean activity when the animals were in dark chambers. These data represent hourly determinations for 29 consecutive days. The mean values for seven animals for each hour were placed in the table under the hour on which the determination ended.

[3] All graphs in this paper are plotted from sliding averages of three adjacent values. For example, the averages of the 2, 3, and 4 P. M. columns were averaged to give the 3 P.M. value. This technique is useful for smoothing curves and shows trends more clearly.

TABLE I

Locomotor activity in the salamander (min./hr.)

	A.M.												P.M.											
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
May 14	3.6	1.9	7.9	1.9	0	0.3	5.7	5.7	6.0	1.1	6.7	5.0	4.0	0	0.7	1.4	1.4	5.6	1.0	8.6	12.9	8.6	6.4	0.3
15	0.4	5.0	9.6	0.3	0.3	5.7	0.3	0.4	4.3	0.7	6.7	5.0	0.3	0.3	0.7	3.6	6.0	12.1	15.7	22.4	5.7	11.7	3.6	7.4
16	10.7	11.0	7.1	5.0	11.1	7.1	0	0.6	1.7	0.3	1.4	1.4	6.1	6.2	3.3	7.0	7.0	8.5	5.8	0.7	8.6	6.7	11.0	22.1
17	10.7	8.6	4.9	7.9	2.1	3.1	0	4.3	5.0	4.6	0.8	4.6	1.4	0	0.3	0	2.1	0.4	3.6	5.7	1.4	0	7.1	7.9
18	4.6	5.0	8.6	7.4	0	5.0	8.9	1.0	4.3	4.3	2.4	0.3	8.6	10.0	3.3	1.0	0	0	16.7	9.7	5.3	2.4	8.9	8.6
19	8.4	11.0	8.6	0	2.1	2.9	0	7.1	1.4	0	3.7	0	0	0	0.7	0	2.0	7.0	1.5	4.3	5.0	9.3	8.0	4.0
20	4.3	1.9	4.4	12.9	5.3	7.9	16.4	8.1	1.7	2.9	0.7	8.6	1.4	0.3	0	0	0.7	7.1	5.3	8.6	0.4	0	1.9	13.6
21	9.3	7.9	5.0	8.6	12.9	13.6	9.3	27.9	16.4	10.0	0	0.4	8.6	7.9	0	0	0	0	0	0	4.6	10.0	8.9	10.7
22	8.9	15.0	17.0	8.6	11.4	9.3	2.9	3.6	3.6	0.3	4.5	0.8	0.5	5.7	1.4	0.7	0	6.4	6.4	9.3	11.1	8.6	7.1	16.0
23	12.1	7.9	8.6	2.1	2.4	6.0	0.7	11.0	10.0	7.5	4.2	9.3	2.6	2.9	7.9	5.8	0.3	0.7	5.7	7.6	12.5	1.2	0.7	0
24	4.1	0	4.3	9.3	5.0	7.4	9.4	0.6	6.4	0.4	0.3	2.0	0	8.3	10.7	2.1	0.6	2.4	17.4	13.2	4.3	12.1	11.4	0
25	0	1.7	3.6	8.6	5.7	5.0	10.7	4.4	0	6.4	0.3	4.3	0	0	0	0	8.6	8.6	15.3	10.3	18.3	25.0	11.0	19.3
26	17.4	19.3	6.4	0.4	13.6	14.6	4.3	0	0.7	0.3	12.5	14.2	0	2.9	7.9	0.7	16.6	20.0	20.3	17.9	23.1	20.0	15.0	23.6
27	4.3	0	3.1	12.9	14.3	8.9	8.9	0.3	6.4	0	0	3.1	12.5	10.0	6.7	8.9	12.1	1.4	13.6	15.3	2.1	12.5	4.2	10.8
28	16.4	13.2	7.1	2.4	6.7	0	0.3	0	0.7	1.4	1.0	5.0	9.3	9.3	12.9	10.0	0	0	19.2	16.7	14.6	12.1	6.9	5.3
29	13.9	31.4	18.1	12.9	8.6	7.7	5.0	14.3	11.3	8.0	3.3	7.9	10.0	0.7	4.3	8.6	1.4	7.1	5.7	10.0	7.9	17.1	5.0	10.7
30	13.6	2.1	6.1	8.6	11.4	4.6	0	8.6	7.1	7.9	4.3	18.3	17.5	2.8	0	0	0	0	1.4	0.7	0	0.8	0	11.6
31	17.1	13.9	2.1	10.7	7.1	3.8	8.6	16.4	5.7	0.7	5.0	13.6	6.0	3.8	12.1	2.1	15.0	20.0	7.5	7.9	0	0	0	0
June 1	4.2	12.9	12.1	4.3	3.8	2.1	7.1	13.9	11.7	13.9	5.0	1.4	1.4	2.1	5.3	0	5.7	3.6	0	0	2.9	2.8	11.7	10.0
2	3.3	0	13.6	8.6	1.4	0	14.3	11.4	10.3	14.3	2.9	3.6	7.9	3.3	17.5	10.3	11.4	8.6	5.3	0.7	3.6	3.6	0	5.0
3	5.7	8.1	10.7	6.4	9.6	2.1	4.3	15.7	6.4	0	0	0	13.6	10.0	2.9	0	0	2.9	8.9	1.7	0	0	4.2	5.0
4	0	19.3	11.4	2.9	2.1	2.9	13.6	17.1	18.0	18.3	8.8	2.0	0	0	7.1	7.1	13.6	14.3	9.3	8.6	3.6	0	5.7	0
5	4.3	5.3	0	0	7.1	22.9	12.1	14.3	2.1	5.3	8.6	8.6	5.3	8.3	5.3	0	0	0.3	0	2.1	25.6	16.2	4.2	8.6
6	4.6	2,1	6.4	1.4	4.6	6.4	4.3	17.9	10.7	9.1	2.9	3.3	0.3	0	5.7	0	0.6	0	0	0	3.6	8.6	9.6	9.5
7	3.3	10.0	14.3	9.3	3.6	3.6	0	0	0	5.3	9.3	8.6	18.6	10.0	10.8	10.0	15.0	21.4	8.6	0.8	0	9.2	15.0	6.0
8	8.6	3.6	0	0	5.7	18.6	12.1	16.4	5.7	6.4	0	1.4	9.3	3.6	5.8	2.8	0	0	0	2.1	7.9	18.6	15.0	1.4
9	2.1	0.7	12.9	8.6	5.7	0.7	0.7	4.3	3.8	0	0	0.3	1.0	9.5	5.8	0	1.2	0	6.7	0	2.1	0.7	18.9	18.6
10	4.6	7.1	15.0	10.0	3.6	0.3	3.6	3.0	3.1	6.2	3.7	0.2	3.0	1.0	0.3	0.9	4.3	2.9	2.9	6.4	13.6	10.0	17.5	12.8
11	15.0	11.4	5.7	0.7	2.1	2.1	1.9	0.3	0	3.6	6.4	0.7	0.3	0.2	1.0	0.3	3.1	0	5.7	7.1	5.7	1.4	0	11.4

The data were first analyzed to find the mean activity for each hour of the day. All 24 columns were averaged and the results are plotted in Figure 1, B. The form of this variation is similar, in a general way, to that obtained when the animals were exposed to alternating light and darkness. There are conspicuous differences between them, however, and these will be discussed later.

Lunar analysis

The data of Table I were inspected for a lunar influence before they were subjected to a detailed analysis. First the data for each day were plotted, each day under the preceding one, and then a line representing lunar zenith was drawn across the plots, intersecting the abscissae at the time of zenith. Since the moon reaches zenith for any given locality approximately 50 minutes later each day, the line representing zenith intersected the time scales about 50 minutes later with each successive day.

Upon close examination of these plots, it was seen that the activity pattern for each day had certain unique variations, but generally bore a similarity to the mean pattern for the 29 days. However, it was noted that when zenith occurred at times of usually moderate or high activity the level of activity was generally low for a few hours before and after the time of zenith. That is to say, when zenith occurred during the "night hours," which were usually the times of greatest activity, depression of activity resulted.

On the starting day of this 29-day study, May 14th, the moon was in third, or last, quarter and thus the time of zenith was approximately 6 A.M. On May 21st new moon occurred with the time of zenith at noon. First quarter, with zenith at approximately 6 P.M., was on the 28th of May and full moon, with zenith at midnight, occurred on June 5th.

It may be postulated that the mean activity for any one day would tend to be high if zenith occurred during the times of normally low activity and would tend to be lower if zenith occurred during the times of high activity. Therefore, if one plots the mean activity for each day for one lunar month, the resulting curve should be essentially the inverse of the mean hourly activity curve, provided the lunar effect is operative in the postulated manner. A comparison of Figure 2, which shows the daily means for a lunar month, with Figures 1, A or 1, B, the hourly mean curves, bears out this hypothesis. This would then suggest strongly that a lunar zenith effect is operative; it is a depressive effect, and the time at which zenith occurs does indeed appear to determine to some extent the mean activity for any one day.

A second method of demonstrating the presence of an apparent lunar modification of the diurnal rhythm was applied in the following manner. If the hourly averages of the days between third quarter and new moon are found, a pattern of activity variation essentially like the average for the entire 29 days should result, since little depressive influence should be in evidence during this time. Figure 3, A, shows the means for each hour of the day for the seven days of that period, and it will be seen that this pattern is very similar to that for the entire 29-day period. Likewise, the hourly means for the succeeding seven days, May 21st to 27th, that is, from new moon to first quarter, should also be similar to the means for the entire 29 days and similar to the pattern for the preceding seven days. Upon examination of Figure 3, B, it will be seen that this is true.

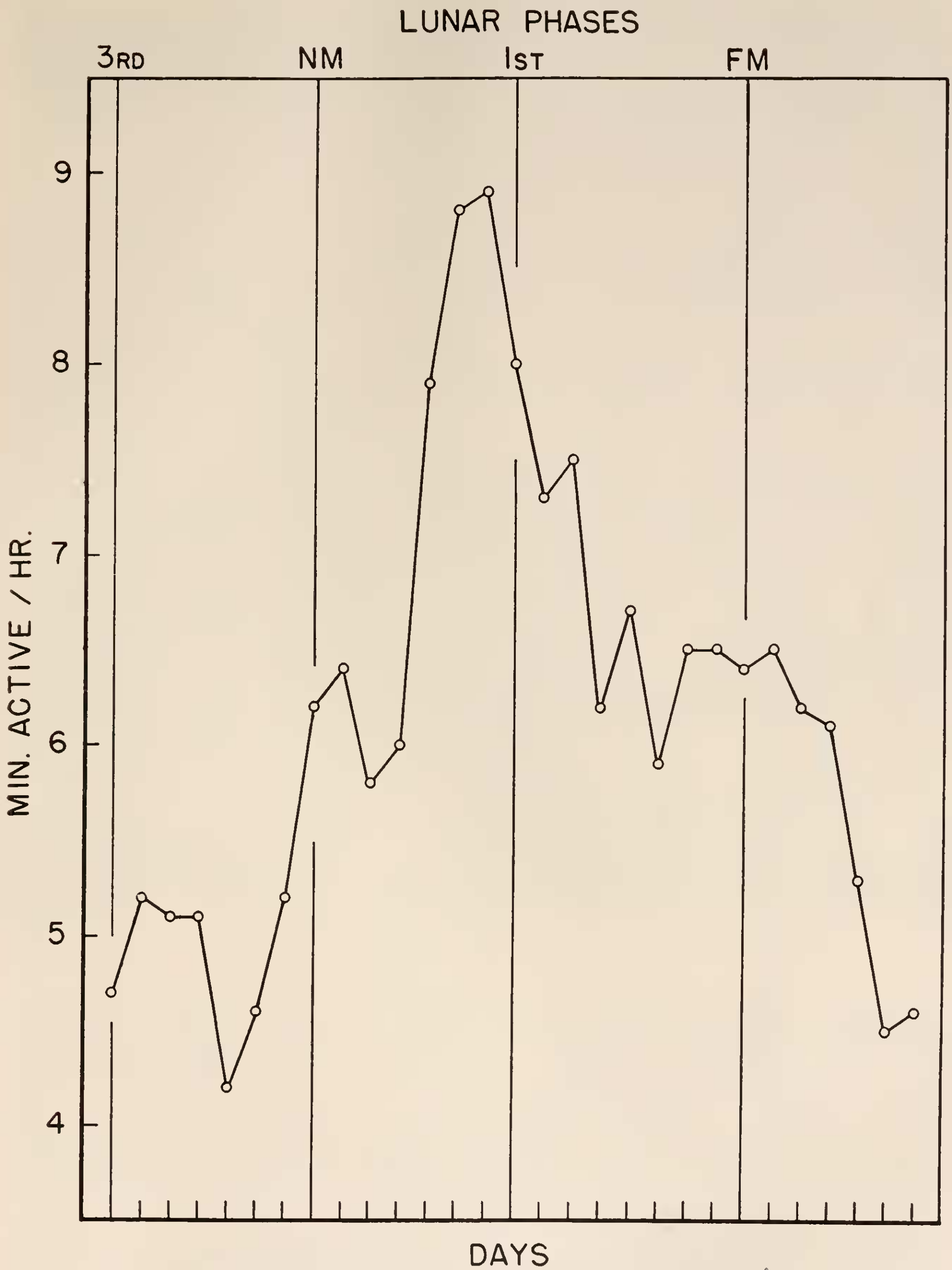

FIGURE 2. The average activity per day for seven salamanders over a 29-day period. The approximate times of the lunar quarters are indicated.

During the next eight days, however, the time of zenith moved from about 6 P.M. to near midnight. Any zenith-associated depressive influence should be very evident during this period. Figure 3, C shows that for these days the lowest levels of activity occurred between 6 P.M. and midnight. Evident, also, in this figure is

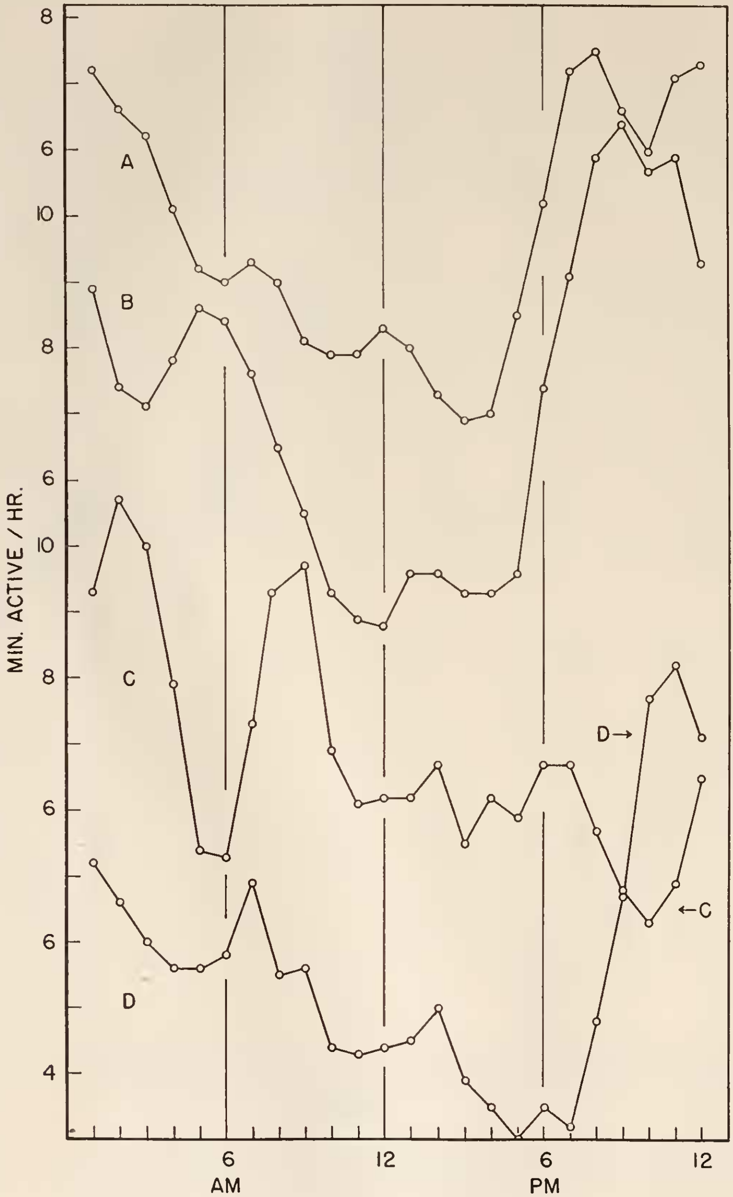

FIGURE 3. The average hourly activity of seven salamanders. A. May 14–20 (3rd quarter-New Moon). B. May 21–27 (New Moon-1st quarter). C. May 28-June 4 (1st quarter-Full Moon). D. June 5–11 (Full Moon-3rd quarter).

a low at 6 A.M. This was largely caused by a low average value of 2.9 at 6 A.M. Consequently, the sliding averages in the region around 6 A.M. are affected. Its significance is unknown.

Finally, the hourly averages for the last quarter of the lunar month are shown in Figure 3, D. Once again, depression appears evident, this time between 1 and 6 A.M., the range over which zenith occurred during the final seven days of this study.

The third method for showing the presence of a lunar rhythm is that which has been employed with much success by Brown (Brown, Bennett and Webb, 1954). For the purposes of this analytical technique, one may visualize the times of lunar zenith, and other corresponding lunar positions, as diagonals running downward and from left to right across the daily rows of data in Table I. As pointed out earlier, the time at which lunar zenith occurs for any one location is later with each successive day by about 50 minutes. Thus, the lunar day is of about 24.8 hours duration. A given lunar position completes one diagonal crossing of all the 24-hour vertical columns in about 29 days.[4]

Any lunar-associated influence may be made apparent if the corresponding lunar positions are aligned in vertical columns. Such a manipulation will serve also to "neutralize" the diurnal rhythm. To accomplish this, the day-by-day data of Table I were shifted to the left an average of about 50 minutes with respect to the preceding day. That is, day 1 was left in its normal hourly relationship, as was day 2, also. Then days 3 to 7 were each moved one hour to the left with respect to the clock hours of the preceding days. The data of day 8 were kept synchronized with those of day 7, but days 9 to 13 were each shifted one hour further to the left, and so on throughout the 29 days of data. Twenty-four vertical columns were retained by transposing, in sequence, the data which extended to the left beyond the first value of day 1 to the right side of the table.

Figure 1, C, shows the results of this analysis. Since zenith occurred at approximately 6 A.M. on the initial day of this experiment and all zeniths of the succeeding days were aligned with it, the position of zenith is indicated in the sixth column in Figure 1, C. This analysis provides further evidence that a depressive effect, which modulates the diurnal rhythm, is associated with the time of lunar zenith.

Discussion

Upon comparison, it will be observed that the amplitude of the average cycle represented in Figure 1, A is about twice that shown by Figure 1, B. This difference may be explained upon the basis of two possible reasons, both of which may apply. When the animals are in alternating light and darkness (Fig. 1, A), the persistent rhythm may be amplified due to a direct influence of light intensity on locomotion. In continuous darkness the rhythm may fail to attain fullest expression, being "damped," but maintaining essentially the same frequency.

A second contributing cause for the difference in amplitude, and one which, in addition, may explain the occurrence of the minor minimum around midnight in Figure 1, A, could be that, in the period represented, only the first half of the A.M. hours, approximately, were subjected to the postulated depressive lunar influence, whereas all hours of the period represented by Figure 1, B were exposed to this

[4] The synodical lunar month, the period from one new moon to the next, has a mean length of 29 days, 12 hours, 44 minutes, and 2.8 seconds.

influence. Thus, Figure 1, B presents the average diurnal rhythm with the lunar influence "neutralized," but nevertheless with lowered amplitude caused by the lunar depression. Figure 1, A is possibly distorted, in part, by the lunar influence, but most of its values were little affected by lunar depression.

The rhythm of locomotor activity, as determined in this experiment under constant laboratory conditions, is undoubtedly similar to the variations in activity of this salamander in nature. Park, Lockett and Myers (1931) note that in the forest this salamander apparently passes the day beneath logs and stones and becomes active by 8:45 P.M.

The possession of a rhythm that assists in regulating the activity of the salamander, as described here, may be of supreme importance for the survival of the animal. As we have seen, it appears to have an activity rhythm that is determined by two components: (1) the diurnal activity pattern, which tends to keep activity minimal during the daylight hours and maximal during the night hours, and (2) the lunar modulation, which alters the diurnal pattern so as to minimize activity on moonlit nights. Thus, the inference is that the animal forays out of its hiding niche on nights when there is little or no moonlight and, hence, when there is less exposure to predators.

Since the period of study extended through only one lunar cycle, it cannot be unequivocably stated that the lunar-frequency modulation constitutes a lunar rhythm, but due to its close correlation with the lunar cycle and the similarity of this modulation with lunar rhythms that have previously been described (*cf.* Brown, Freeland and Ralph, 1955), it appears very likely that it is a lunar rhythm.

Though there appears to be a causal relationship between the lunar cycle and the lunar modification, there need be no direct inductive influence of the moon affecting the organisms. Diurnal rhythms show a causal relationship to the day-night cycle, but, as several studies have shown, the phase relationships of rhythms to the day-night cycle need not be fixed. Thus, the apparent "influence" of the moon on the activity rhythm may be only a persisting behavioral pattern that continues after the inductive influence of the moon is removed. Just as the 24-hour solar cycle may be impressed upon the activity pattern, likewise the 24.8-hour lunar cycle may also be impressed upon the pattern. The two frequencies together would appear largely to determine the overt expression of activity.

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Summary

1. The salamander, *Plethodon cinereus,* shows a diurnal rhythm of locomotor activity, both in alternating 12-hour periods of light and darkness, and in continuous darkness.

2. The diurnal rhythm is strongly modified by a depressive influence that is apparently associated with the time of lunar zenith.

3. The activity of the animal at any given time is a function of the diurnal and lunar influences operative at that time.

4. The significance of the rhythm to the animal in nature is discussed.

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